CLAIMS:-

1. A method of producing a DNA molecule wherein mRNA transcribed from the DNA molecule forms hairpin RNA (hRNA), the method comprising:

(i) synthesizing a first DNA strand comprising in order a first sequence, a random sequence and a second sequence, wherein nucleotides at the 3' end of the second sequence are complementary to nucleotides at the 5' end of the second sequence such that the second sequence forms a stem loop;

(ii) synthesizing a complementary DNA strand extending from the stem loop using a DNA polymerase, the complementary DNA strand being complementary to the first sequence and the random sequence so as to form hairpin DNA;

(iii) denaturing the hairpin DNA to form a single DNA strand; and

(iv) adding a primer which hybridises to the complement of the first sequence and DNA polymerase to synthesize double stranded DNA.

2. A method according to claim 1 wherein a deoxyuracil nucleotide is included in the first sequence and prior to addition of the primer the single DNA strand is depurinated and β-eliminated.

3. A method according to claim 1 or claim 2 wherein the first DNA strand includes a restriction enzyme site.

4. A method according to any one of claims 1 to 3 wherein the random sequence is at least 19 base pairs, preferably 19 to about 30 base pairs, more preferably from 19 to 25 base pairs in length.

5. A method according to claim 4 wherein the random sequence is 19 base pairs in length.

6. A method according to any one of claims 1 to 5 wherein the double stranded DNA is cloned into an expression vector under the control of a U6 snRNA, H1 or T7 promoter.

7. A method according to claim 6 wherein the double stranded DNA is cloned into an expression vector under the control of a U6 snRNA promoter.

8. A method of preparing an expression vector, expression of which produces double stranded RNA (dsRNA), the method comprising:

(i) synthesizing a first DNA strand comprising in order at least four consecutive adenosine nucleotides, a random sequence, at least four consecutive thymidine nucleotides and a primer binding site;

(ii) annealing a primer to the primer binding site and synthesizing a second DNA strand which is substantially complementary to the first DNA strand and forms double stranded DNA; and

(iii) cloning the double stranded DNA into an expression vector between two convergent promoters.

9. A method according to claim 8 wherein the random sequence is at least 19 base pairs, preferably 19 to about 30 base pairs, more preferably from 19 to 25 base pairs in length.

10. A method according to claim 9 wherein the random sequence is 19 base pairs in length.

11. A method according to any one of claims 8 to 10 wherein the double stranded DNA is cloned into an expression vector under the control of a U6 snRNA, H1 or T7 promoter.

12. A method according to claim 11 wherein the double stranded DNA is cloned into an expression vector between two convergent U6 snRNA promoters.

13. A method for determining a function of a gene, the method comprising:

(i) synthesizing a first DNA strand comprising in order a first sequence, a random sequence and a second sequence, wherein nucleotides at the 3' end of the second sequence are complementary to nucleotides at the 5' end of the second sequence such that the second sequence forms a stem loop;

(ii) synthesizing a complementary DNA strand extending from the stem loop using a DNA polymerase, the complementary DNA strand being complementary to the first region and the random sequence so as to form hairpin DNA;

(iii) denaturing the hairpin DNA to form a single DNA strand;

(iv) adding a primer which hybridises to the complement of the first sequence and DNA polymerase to synthesize double stranded DNA;

(v) cloning the double stranded DNA into an expression vector wherein the double stranded DNA is under the control of a promoter;

(vi) transfecting an effective amount of the expression vector into a cell under conditions permitting transcription of the double stranded DNA to produce a transfected cell; and

(vii) detecting one or more changes in the transfected cell relative to a control cell.

14. A method according to claim 13 wherein a deoxyuracil nucleotide is included in the first sequence and prior to addition of the primer the single DNA strand is depurinated and β-eliminated.

15. A method according to claim 13 or claim 14 wherein the first DNA strand includes a restriction enzyme site.

16. A method according to any one of claims 13 to 15 wherein the random sequence is at least 19 base pairs, preferably 19 to about 30 base pairs, more preferably from 19 to 25 base pairs in length.

17. A method according to claim 16 wherein the random sequence is 19 base pairs in length.

18. A method according to any one of claims 13 to 17 wherein the double stranded DNA is cloned into an expression vector under the control of a U6 snRNA, H1 or T7 promoter.

19. A method according to claim 18 wherein the double stranded DNA is cloned into an expression vector under the control of a U6 snRNA promoter.

20. A method for determining a function of a gene, the method comprising:

(i) synthesizing a first DNA strand comprising in order at least four consecutive adenosine nucleotides, a random sequence, at least four consecutive thymidine nucleotides and a primer binding site;

(ii) annealing a primer to the primer binding site and synthesizing a second DNA strand which is substantially complementary to the first DNA strand and forms double stranded DNA;

(iii) cloning the double stranded DNA into an expression vector between two convergent promoters;

(iv) transfecting an effective amount of the expression vector into a cell under conditions favouring transcription of the double stranded DNA to produce a transfected cell; and

(v) detecting one or more changes in the transfected cell relative to a control cell.

21. A method according to claim 20 wherein the random sequence is about 19 to about 30 base pairs in length, preferably from 19 to 25 base pairs in length.

22. A method according to claim 21 wherein the random sequence is 19 base pairs in length.

23. A method according to any one of claims 20 to 22 wherein the double stranded DNA is cloned into an expression vector under the control of a U6 snRNA, H1 or T7 promoter.

24. A method according to claim 23 wherein the double stranded DNA is cloned into an expression vector between two convergent U6 snRNA promoters.

25. An expression vector for use in suppressing expression of a target gene, the vector comprising a pair of convergent promoters and a DNA molecule positioned therebetween, the DNA molecule comprising a target-specific sequence flanked by two directional transcription terminators, the target-specific sequence comprising a sequence of at least 14 nucleotides having at least 90% identity to a segment of the target gene.

26. An expression vector according to claim 25 wherein the target-specific sequence is at least 19 base pairs, preferably 19 to about 30 base pairs, more preferably from 19 to 25 base pairs in length.

27. An expression vector according to claim 26 wherein the target-specific sequence is 19 base pairs in length.

28. An expression vector according to any one of claims 25 to 27 wherein the target-specific sequence has at least 95% identity, and more preferably is identical, to a segment of the target gene.

29. An expression vector according to any one of claims 25 to 28 wherein the expression vector is a retroviral expression vector.

30. An expression vector according to any one of claims 25 to 29 wherein the convergent promoters are U6 snRNA, H1 or T7 promoters.

31. An expression vector according to any one of claims 25 to 30 wherein the convergent promoters are U6 snRNA promoters.

32. A method for determining a function of a target gene, the method comprising:

(i) preparing an expression vector comprising a pair of convergent promoters and a DNA molecule positioned therebetween, the DNA molecule comprising a target-specific sequence flanked by two directional transcription terminators, the target-specific sequence comprising a sequence of at least 14 nucleotides having at least 90% identity to a segment of the target gene;

(ii) transfecting an effective amount of the siRNA expression vector into a cell to produce a transfected cell; and

(iii) detecting one or more phenotypic changes in the transfected cell relative to a control cell.

33. A method according to claim 32 wherein the target-specific sequence is at least 19 base pairs, preferably 19 to about 30 base pairs, more preferably from 19 to 25 base pairs in length.

34. A method according to claim 33 wherein the target-specific sequence is 19 base pairs in length.

35. A method according to any one of claims 32 to 34 wherein the target-specific sequence has at least 95% identity, and more preferably is identical, to a segment of the target gene.

36. A method according to any one of claims 32 to 35 wherein the expression vector is a retroviral expression vector.

37. A method according to any one of claims 32 to 36 wherein the convergent promoters are U6 snRNA, H1 or T7 promoters.

38. A method according to any one of claims 32 to 37 wherein the convergent promoters are U6 snRNA promoters.

39. A method of inhibiting expression of a target gene in a cell, the method comprising introducing into the cell an expression vector according any one of claims 25 to 31.

40. A method of producing a library of DNA molecules wherein mRNA transcribed from the DNA molecules forms hairpin RNA (hRNA) molecules, the method comprising:

(i) preparing a library of double stranded DNA fragments;

(ii) ligating hairpin DNA to the DNA fragments from step (i);

(iii) ligating a double stranded DNA adaptor to the DNA from step (ii), wherein the DNA adaptor includes a primer binding site;

(iv) denaturing the DNA from step (iii) to form a library of single DNA strands; and

(v) adding a primer which hybridises to the primer binding site and DNA polymerase to synthesize double stranded DNA thereby producing a library of double stranded DNA molecules.

41. A method according to claim 40 wherein the library of double stranded DNA fragments is prepared by digestion of DNA.

42. A method according to claim 41 wherein the DNA is a gene.

43. A method according to claim 41 wherein the DNA is a genome.

44. A method according to claim 41 wherein the DNA is a cDNA library.

45. A method according to any one of claims 41 to 44 wherein the digestion is with DNAseI.

46. A method according to any one of claims 40 to 45 wherein the double stranded DNA molecules are cloned into expression vectors under the control of a promoter selected from the group consisting of U6 snRNA, H1 and T7.

47. A method according to claim 46 wherein the double stranded DNA molecules are cloned into expression vectors under the control of a U6 snRNA promoter.

48. A method of preparing a library of expression vectors, expression of which produces double stranded RNA (dsRNA) molecules, the method comprising:

(i) preparing a library of double stranded DNA fragments;

(ii) ligating a double stranded DNA adaptor to each end of the DNA fragments from step (i), wherein the sequence of the DNA adaptor comprises at least four consecutive adenosine nucleotides at the 3' end; and

(iii) cloning the double stranded DNA from step (ii) into an expression vector between two convergent promoters.

49. A method according to claim 48 wherein the library of double stranded DNA fragments is prepared by digestion of DNA.

50. A method according to claim 49 wherein the DNA is a gene.

51. A method according to claim 49 wherein the DNA is a genome.

52. A method according to claim 49 wherein the DNA is a cDNA library.

53. A method according to any one of claims 49 to 52 wherein the digestion is with DNAseI.

54. A method according to any one of claims 48 to 53 wherein the double stranded DNA molecules are cloned into expression vectors under the control of a promoter selected from the group consisting of U6 snRNA, H1 and T7.

55. A method according to claim 54 wherein the double stranded DNA molecules are cloned into expression vectors under the control of a U6 snRNA promoter.

56. A method of producing a library of DNA molecules wherein mRNA transcribed from the DNA molecules forms hairpin RNA (hRNA) molecules, the method comprising:

(i) preparing a pool of mRNA;

(ii) adding an enzyme to the pool of mRNA, wherein the enzyme reverse transcribes the mRNA to form cDNA and degrades the mRNA;

(iii) allowing the cDNA from step (ii) to form a hairpin loop;

(iv) synthesising a second strand using the hairpin loop as a priming point for reverse transcriptase;

(v) denaturing the DNA from step (iv) to form single stranded DNA; and

(vi) adding DNA polymerase to synthesize double stranded DNA thereby producing a library of double stranded DNA molecules.

57. A method according to claim 56 wherein the enzyme in step (ii) is AMV reverse transcriptase.

58. A method according to claim 56 or 57 wherein the double stranded DNA molecules are cloned into expression vectors under the control of a promoter selected from the group consisting of U6 snRNA, H1 and T7.

59. A method according to claim 58 wherein the double stranded DNA molecules are cloned into expression vectors under the control of a U6 snRNA promoter.